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# A first-generation physiologically based pharmacokinetic (PBPK) model of alpha-tocopherol in human influenza vaccine adjuvant

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ABSTRACT

Alpha (α)-tocopherol is a component of a new generation of squalene-containing oil-in-water (SQ/W) emulsion adjuvants that have been licensed for use in certain influenza vaccines. Since regulatory pharmacokinetic studies are not routinely required for influenza vaccines, the *in vivo* fate of this vaccine constituent is largely unknown. In this study, we constructed a physiologically based pharmacokinetic (PBPK) model for emulsified α-tocopherol in human adults and infants. An independent sheep PBPK model was also developed to inform the local preferential lymphatic transfer and for the purpose of model evaluation. The PBPK model predicts that α-tocopherol will be removed from the injection site within 24 h and rapidly transfer predominantly into draining lymph nodes. A much lower concentration of α-tocopherol was estimated to peak in plasma within 8 h. Any systemically absorbed α-tocopherol was predicted to accumulate slowly in adipose tissue, but not in other tissues. Model evaluation and uncertainty analyses indicated acceptable fit, with the fraction of dose taken up into the lymphatics as most influential on plasma concentration. In summary, this study estimates the *in vivo* fate of α-tocopherol in adjuvanted influenza vaccine, may be relevant in explaining its immunodynamics in humans, and informs current regulatory risk-benefit analyses.

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## 1. Introduction

Vaccination continues to play a highly important role in protecting and promoting public health. Numerous approaches have been exploited in the development of successful vaccines, and these involve the use of vaccine adjuvants. Adjuvants are components of certain vaccines that augment the magnitude and/or modulate the quality of the immune response (Koff et al., 2013; Levitz and Golenbock, 2012; Pulendran and Ahmed, 2011; Rappuoli, 2011). Aluminum salts are the oldest and the most widely used vaccine adjuvants, but in recent years a new class of vaccine adjuvants has emerged: the squalene-containing oil-in-water (SQ/W) emulsion adjuvants. This class includes, among others, MF59® and AS03®, which are components of certain influenza vaccines licensed by the European Medicines Authority (EMA) or the US Food and Drug Administration (USFDA) (Garcon et al., 2012; Gasparini et al., 2012; Levitz and Golenbock, 2012; O'Hagan et al., 2011). Interest in the use of squalene-containing adjuvants has grown following the H1N1 pandemic as a means of sparing antigen while increasing immunogenicity.

AS03® is a new-generation squalene-containing emulsion adjuvant that contains α-tocopherol (11.86 mg) and squalene (10.69 mg) in the oil phase, emulsified by polysorbate 80 (4.86 mg), and a continuous aqueous phase of phosphate-buffered saline (Garcon et al., 2012). Alpha-tocopherol ($C_{29}H_{50}O_2$) is also the biologically active form of vitamin E, an essential, dietary lipid-soluble antioxidant responsible for protecting cell membranes against lipid peroxidation (Biesalski, 2009). The precise mechanism by which SQ/W emulsions potentiate the immune response is not completely understood. However, experimental evidence with the adjuvant MF59® suggests enhanced immune cell recruitment, antigen uptake and upregulation of several cytokines and chemokines at the site of administration following intramuscular (IM) injection (Calabro et al., 2011; Mosca et al., 2008; O'Hagan et al., 2012). Experimental studies in rodents and model-based pharmacokinetic studies in humans for SQ/W emulsions also suggest the absence of a local depot effect and rapid decay of the emulsion from the site of injection (Dupuis et al., 1999; Ott et al., 1995; Tegenge and Mitkus, 2013). The addition of α-tocopherol into the oil phase of SQ/W emulsions has been shown to influence both

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the kinetics and the level of some cytokines at injection sites (Garcon et al., 2012; Morel et al., 2011).

The pharmacokinetics of α-tocopherol following oral administration has been extensively studied in humans (Blomstrand and Forsgren, 1968; Ferslew, et al. 1993; Jeanes, et al. 2005; Kelleher and Losowsky, 1968; Traber et al., 1994). Those studies have generally shown that α-tocopherol is mainly absorbed via the lacteals (lymphatic vessels in the small intestine), transported in plasma via lipoprotein binding, and cleared from plasma with a half-life of about 48 h. However, the pharmacokinetic database for α-tocopherol solutions administered intramuscularly to humans appears to be limited to only two published studies. A study in premature neonates indicated that IM injection of α-tocopherol in a *non-emulsified* alcoholic solution peaked in plasma within 4 h and was cleared slowly with a half-life of 44 h (Colburn and Ehrenkranz, 1983). The second study used α-tocopherol *acetate* in colloidal or olive oil solution for IM injection in premature neonates (Italian Collaborative Group on Preterm Delivery, 1991). However, the emulsified alcohol, α-tocopherol, is the relevant form utilized in influenza vaccine adjuvants and its pharmacokinetics has not been studied experimentally. The former observation is crucial to adopting an appropriate modeling strategy, because the formulation in which α-tocopherol is delivered (e.g., oil-in-water vs. water-in-oil emulsion or alcoholic vs. oil solution), as well as the species of α-tocopherol (e.g., alcohol vs. ester) have been long known to significantly affect the uptake of this compound into blood, as well as its tissue distribution (Bateman and Uccellini, 1985; Njeru et al., 1994; Schmandke and Schmidt, 1965).

Thus, we have carefully searched the literature database for the vaccine-relevant form of α-tocopherol (i.e. the alcohol form rather than ester) and formulation (i.e. O/W emulsion rather than free alcoholic or oil solution) and are aware of only two published pharmacokinetic studies, in any mammalian species, of α-tocopherol delivered in an O/W emulsion and administered IM, i.e., similar to the human vaccination scenario for influenza vaccines. Because the identified studies were conducted in sheep (Hidiroglou and Karpinski, 1991; Njeru et al., 1994), direct quantitative extrapolation of the results to the human situation would be highly uncertain. However, in such a scenario, physiologically-based pharmacokinetic (PBPK) modeling based on rational, well-informed assumptions can provide important predictions of the time course and tissue distribution in the relevant target species (humans), especially when experimental data in that species are lacking. In addition, since experimental pharmacokinetic studies in humans are not currently required for vaccine licensing across the world (Sun et al., 2012; WHO, 2005; Wolf et al., 2010), PBPK models informed by relevant animal data serve as the basis for our predictions of the disposition of novel vaccine adjuvants (like MF59® and AS03®) in humans. Since they provide quantitative predictions of target concentration, they also in turn may inform the mechanism of action of these new products and contribute to a broader understanding of both benefit and risk in human subjects.

The purpose of this study was two-fold: (1) to develop a generic, flexible PBPK model for evaluation of the *in vivo* fate of novel vaccine adjuvants, and (2) to estimate the biodistribution of, specifically, α-tocopherol, in humans following a single dose of squalene-containing adjuvant. We present the results of PBPK model predictions that describe the disposition of α-tocopherol in an SQ/W emulsion at the site of injection and draining lymph nodes in both human adults and infants. Furthermore, the PBPK model predicts the distal tissue distribution of α-tocopherol following injection of adjuvanted influenza vaccine. Finally, we compared vaccine-derived α-tocopherol concentration in human plasma and other tissues with that of background concentrations in plasma and selected tissue, as a useful first step in a quantitative risk analysis for this class of vaccine adjuvants.

## 2. Methods

### 2.1. PBPK model development and compound-specific assumptions

The generic PBPK model for vaccine adjuvant consisting of local and distal tissues is depicted in Fig. 1A. Based on the adjuvant system AS03®, the oil phase of the emulsion is composed of squalene and α-tocopherol. The oil phase is emulsified with the surfactant polysorbate 80 and has an average droplet size of about 180 nm (Garcon et al., 2012), comparable with the adjuvant MF59®. The PBPK model considered transport to both blood and draining lymph nodes from injection site muscle (Fig. 1B). However, because of the large size of the emulsion (Garcon et al., 2012), the type of local exposure (primarily interstitial during intramuscular injection), its highly lipophilic nature (log $P = 12$); Gershkovich and Hoffman, 2005), and the known lymphatic absorption of oral α-tocopherol and lipophilic drugs as emulsions in association with chylomicrons (Blomstrand and Forsgren, 1968), we assumed preferential transport into lymphatics, rather than into blood following local injection of emulsified α-tocopherol. The *in vivo* stability of O/W emulsions is largely unknown; however, limited cracking would be expected following IM injection as a result of degradation or dissociation of the surfactant used to stabilize the emulsion (Bollinger, 1970; Kalvodova, 2010; van Tellingen et al. 1999). Hence, we also incorporated an estimate of cracking of the emulsion ($k_c$) at the site of injection and within the draining lymph nodes similar to our recently published squalene model (Tegenge and Mitkus, 2013).

The disposition of the emulsion in the injection site muscle and draining lymph nodes was described schematically (Fig. 1), as well as mathematically (Supplementary 1). Because of rapid esterase degradation of the polysorbate 80 in plasma (van Tellingen et al., 1999), we assumed that any emulsion that reached plasma would instantaneously crack, and the released α-tocopherol will then be transported primarily via binding to lipoproteins. The major lipoprotein that binds and transports α-tocopherol in plasma is low density lipoprotein (LDL) (Behrens et al., 1982; Biesalski, 2009; Bjornson et al., 1976; Davies et al., 1969). Subsequent uptake of α-tocopherol into cells of target tissues is mainly via receptor-mediated endocytosis of LDL (Biesalski, 2009; Thellman and Shireman, 1985), while very limited diffusion of free α-tocopherol into or out of tissues can be expected based on its very high log P. Receptor-mediated uptake of α-tocopherol into selected tissues (liver, spleen, kidney and GIT) was modeled based on the uptake of radiolabeled LDL following binding to the LDL receptor in human tissues (Rudling et al., 1990). Distribution to the rest of the body was modeled for free α-tocopherol under flow-limited and well-stirred conditions. Each tissue compartment was described with a mass-balance differential equation that consisted of a series of tissue concentrations, flow rates and a partition coefficient. Detail of the model code and equations are displayed in Supplementary 1.

### 2.2. Physiological parameters

Based on the results of our previous model for emulsified squalene (Tegenge and Mitkus, 2013), lymphatic flow was modeled only for the injection site leading to the draining lymph nodes then to plasma. Lymphatic flow at the injection site was set at 0.2% of the rate of blood flow to injection site muscle and draining lymph nodes (Swartz, 2001). Tissue volume for human adult (male mean body weight, BW = 73 kg) and 1 year old infant (mean BW = 10 kg) were estimated based on ICRP89, Table 2.8 (ICRP 89, 2002). We obtained the absolute value of cardiac output (CO) for both adult and infant (ICRP 89, 2002). The % CO to each tissue in adult human (ICRP 89, 2002) was used to estimate blood flow for both adult and